Conspecific Gamete Precedence in Speciation: History, Evidence, and a Research Roadmap

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ABSTRACT

Barriers to hybridization can act between gamete release and fertilisation. When an egg donor has simultaneous access to conspecific and heterospecific pollen or sperm, competitive interactions can bias fertilisation towards conspecific gametes, producing conspecific gamete precedence (CGP). Although such biases were first reported more than 250 years ago, CGP remains poorly integrated into the study of speciation. Here, we trace the history of CGP research from Joseph Gottlieb Kölreuter's crossing experiments in the 1760s and review its reported occurrence in plants and animals. Since the 1970s, when CGP was first studied in an explicit speciation framework, the apparent occurrence of CGP has been reported in 93 case studies. However, the strength of this evidence varies because observed paternity can also reflect differences in gamete quantity and viability, non-competitive fertilisation success, and offspring survival. We therefore propose a common experimental and statistical framework for distinguishing CGP from these alternative sources of bias. Finally, we identify opportunities to investigate its mechanisms, evolutionary dynamics, and contribution to reproductive isolation. Standardising how CGP is tested and reported will make studies more comparable and allow its role in speciation to be assessed with greater confidence.

Key words: gametic isolation, conspecific pollen precedence, conspecific sperm precedence, competitive fertilisation, cryptic female choice, pollen competition, sperm competition, speciation history

Introduction

The direct interaction between compatible gametes is a common feature of sexual reproduction in plants and animals. For some sessile organisms, those gametic interactions constitute the only instance where the male and female phenotypes directly interact (Beekman et al., 2016; Bishop & Pemberton, 2006; Tonnabel et al., 2021). They also can play a role in creating reproductive isolation in ways that are both idiosyncratic and similar to the action of prepollination and precopulatory reproductive barriers (Birkhead & Pizzari, 2002; Eberhard, 1996; Garlovsky et al., 2024; Howard et al., 2009; Kekäläinen & Evans, 2018; Pitnick et al., 2009; Tonnabel et al., 2021). For instance, when more than one pollen/sperm donor participates in reproduction, there is scope for pollen/spermatozoa to display offensive strategies that increase a gamete's ability to outcompete other gametes or defensive strategies that prevent future competition (Arnqvist & Rowe, 2005). The reproductive tissues of the egg donor can also exert their preference and either facilitate or block fertilisation by certain types of gametes (Eberhard, 2009). As a consequence of these interacting processes, non-random fertilisation can occur, biasing the paternity of the offspring towards particular individuals.

In hybridizing species, conspecific gamete precedence (CGP) can arise from competitive gametic interactions. This phenomenon occurs when the eggs of an individual that has simultaneous access to pollen/sperm from both the same species (conspecific) and a different species (heterospecific) are preferentially fertilised by gametes from the same species (Howard, 1999). This form of postpollination/postmating prezygotic (PMPZ) isolation is different to non-competitive gametic isolation because it requires the simultaneous presence of conspecific and heterospecific pollen/sperm to be expressed (Coyne & Orr, 2004). Operationally, differences in the fertilisation ability of conspecific and heterospecific gametes in non-competitive crosses form the baseline upon which CGP is tested. Its occurrence has been documented in numerous species and might represent a common reproductive barrier in plants and animals (Garlovsky et al., 2024; Howard, 1999; Howard et al., 2009). Yet, we lack a common framework to test the occurrence of CGP across groups with different reproductive strategies.

Despite constituting a common observation in crossing experiments for more than 250 years, CGP only began to be incorporated into the speciation research agenda in the last few

decades, with different authors using a myriad of terms to describe the same phenomenon (Howard, 1999). Here, using the historical background of the study of CGP, we synthesise current knowledge about its occurrence. First, we distinguish three periods that differ in their pace of research and scientific interests around observations of biases in fertilisation, which ultimately led to the maturation of the CGP idea in the framework of speciation. Second, we document the available evidence for the occurrence of CGP in plants and animals, revealing taxonomic biases and other occurrence patterns. Third, we use the modern definition of CGP to critically assess the standard of evidence for its occurrence while delineating the best practices to design competitive fertilisation experiments. By reviewing this field, we offer a new perspective of the long yet overlooked history of CGP and provide a common framework to propel the full integration of CGP into studies of speciation.

The neglected contributions of Kölreuter and Gärtner

Kölreuter (1763, 1764, 1766) was the first scholar to conduct experiments of pollen competition and reveal the existence of what looks like CGP. As part of a monumental crossing research program to explore the nature of hybridisation in plants (reviewed in Mayr (1986)), Kölreuter carried out numerous crosses where one individual was simultaneously pollinated with pollen from individuals of the same and a different species, and the paternity of the offspring was subsequently inferred through phenotypic similarity between parents and offspring (Table S1). Through these experiments, Kölreuter aimed at demonstrating whether the "semen" [*Samenstaube*] of two pollen donors could blend and produce half-hybrids (Mayr, 1986; Roberts, 1929). Contrary to this widespread belief of his time, the offspring did not have an intermediate phenotype but instead resembled only the conspecific pollen donor in virtually all cases. This was a puzzling finding considering his own observations where the heterospecific pollen was fully capable to produce hybrids in non-competitive crosses. After observing that pollinators commonly deposit both conspecific and heterospecific pollen on stigmas, Kölreuter (1766) proposed a "law of near-relationship" that explained the scarcity of hybrids in nature: "if sufficient quantities of its own and of alien pollen are placed on the stigma, it is only its own pollen that is accepted for this important function; the alien one, however, is totally displaced and excluded from fertilization." [Mayr's (1986) translation.]

Some eighty years after Kölreuter, Gärtner (1849) conducted new competitive pollination experiments. He had already considered the occurrence of CGP a proven

phenomenon and, instead of repeating Kölreuter's experiments, Gärtner focused his efforts on discovering the mechanisms that favoured CGP. He was interested in testing the effect of the order, timing, and quantity of pollen deposition on the fertilisation outcome. Depending on the species, conspecific pollen no longer enjoyed precedence in fertilisation when it was applied 2-5 h after the heterospecific pollen. Motivated by these findings, Gärtner carried out a series of competitive pollination experiments where he made meticulous observations about changes in floral traits for several days after pollen application. He aimed at dissecting when and where in the fertilisation process the heterospecific pollen gained precedence over the conspecific pollen. While reflecting on the occurrence of CGP in nature, whose demonstration Darwin (1859, 1868) attributed to Gärtner, Darwin (1868) recognised its potential role in preventing the formation of plant hybrids and envisioned it could evolve by natural selection to keep the integrity of "a species in process of formation."

However, some of Darwin's later experimental approaches to selective fertilisation reflected interests beyond CGP, including early research on factors other than relatedness as determinants of the outcome of competitive fertilisation. While working with different varieties of self-compatible species, Darwin (1859, 1876) was intrigued by a series of consistent observations seemingly contrary to CGP: "the pollen of a distinct variety having a prepotent effect over a flower's own pollen," even if the foreign pollen was applied 24 h after self-pollen. Darwin also recognised the role of selective fertilisation within species while working on the reproductive patterns of heterostyled plants. In studying this floral polymorphism, Darwin (1868, 1872) observed that "legitimate pollen [from pollen donors with styles of the same length than the egg donor's] is strongly prepotent over illegitimate pollen [vice versa], when both are placed on the same stigma," a result that he considered analogous to CGP. Curiously, Gregor Mendel incidentally conducted competitive pollination experiments in 1869 to refute Darwin's (1868) assertion that more than one pollen grain can participate in fertilisation, but no records of the outcome of those experiments are currently known (van Dijk & Ellis, 2022).

Selective fertilisation studies and revival of interest in speciation research

After a nearly 50-year absence in the literature, competitive fertilisation experiments were performed again as part of what scholars of the time called selective fertilisation studies.

The emphasis in this phase of research was on fertilisation biases in relation to traits of the parental individuals, not their relatedness. These studies were conducted mostly between varieties of economically important plants (Balls, 1919; Collins & Kempton, 1913; Jones, 1918, 1920b, 1920a; Kearney, 1923; Kearney & Harrison, 1924, 1932) and between distantly related broadcast spawning marine invertebrates (Godlewski, 1911; Lillie, 1921; Loeb, 1915). Additional intraspecific studies on selective fertilisation, along with an increasing understanding of inheritance, led early scholars of the twentieth century to both be sceptical about its occurrence and perceive it as "a sort of nightmare to investigators in genetics" because it apparently violated Mendel's laws of segregation and recombination (East, 1919). Nevertheless, one decade later, the occurrence of selective fertilisation in relation to morphological traits was widely acknowledged in both plants and animals (Brieger, 1926; Jones, 1928; King, 1929). In particular, Jones' (1920a, 1922, 1928) work offered thorough insights on the significance of selective fertilisation for evolution, recognising it as an assortative mating-like process that discriminates gametes in relation to their evolutionary relatedness. Despite these burgeoning ideas, and the realisation that gametic interactions can play a role in reproductive isolation between species (Dobzhansky, 1935, 1937), proper studies of CGP were seemingly absent during this period. Instead, the research agenda on selective fertilisation shifted its focus to other processes operating within species.

In an effort to disambiguate different terms previously used to describe selective fertilisation phenomena (see below), Whiting (1935) listed several processes that have been studied under the umbrella of selective fertilisation. By then, it was clear that scholars were mostly interested in cases that represented deviations from Mendelian inheritance, namely transmission ratio distortion. This was easier to study than CGP because it did not require performing competitive crosses and assessing the paternity of the offspring. Instead, those studies conducted non-competitive crosses involving heterozygous individuals for a trait of interest and then scored the trait in the offspring. However, in plants, it was still common to carry out competitive pollination experiments between varieties of the same species to study selective fertilisation in relation to heterostyly (McKenna, 1992; Tseng, 1937) and its application in agriculture through the mentor effect (Stettler, 1968; Stettler & Ager, 1984) and gametophyte selection (Mulcahy, 1971; Ottaviano et al., 1988). Although these studies did not directly address CGP, they increased our mechanistic understanding of differential fertilisation in plants by conducting crosses under different experimental conditions in species that varied in reproductive traits (Ruane, 2009).

Enthusiasm for CGP studies only re-emerged in the 1980s when a general revival of interest in the genetics and ecology of speciation took place, partially facilitated by the emergence of model systems that involved closely related species (Howard, 1999). Although early treatises of plant and animal speciation considered gametic interactions as potential reproductive barriers (Grant, 1971; Mayr, 1963; Stebbins, 1950), they also downplayed the importance of PMPZ barrier in relation to other reproductive barriers because it often failed to completely prevent the production of hybrids in controlled crosses (Howard, 1999; Howard et al., 1998). Furthermore, the inherent inability of typical non-competitive crossing experiments to detect CGP led scholars to overlook CGP for the greater part of the twentieth century (Coyne & Orr, 2004; Howard, 1999; Howard et al., 1998). While Smith (1968, 1970) conducted the first CGP studies just before this period started, his main interest was to measure the genetic differentiation between closely related species. It was Kiang and Hamrick (1978) who first experimentally addressed CGP as a reproductive barrier in plants, followed by Nakano (1985) in insects. Ever since, CGP started to be incorporated in speciation studies of model systems for ecology and evolution (Carlson et al., 2009; Price, 1997; Ramsey et al., 2003; Rieseberg et al., 1995; Ruane & Donohue, 2008). Since the publication of Howard's (1999) influential review on CGP, the number of known CGP case studies has almost tripled (Figure 1; see below). This proliferation in case studies led to an increase in taxonomic breadth and scope, including the first reports of CGP in marine invertebrates (Bierne et al., 2002), fishes (Ludlow & Magurran, 2006), and mammals (Dean & Nachman, 2009; Martín-Coello et al., 2009). However, it seems that the pace of research on CGP has slowed down since 2020.

Semantic confusion and prejudices

Observations of biased fertilisation in relation to relatedness have been described using dozens of different terms for more than one century, which might have contributed to undercount CGP case studies in literature surveys and hinder its early appreciation as a prevalent phenomenon in nature (Howard, 1999). Some of those early terms included prepotency (Darwin, 1859), selective fertilisation (East, 1919), certation (Heribert-Nilsson, 1920), homogamy (Jones, 1920a), gamolysis (Jones, 1928), preponderancy (Kearney & Harrison, 1932), and gametophytic selection (Whiting, 1935). However, the CGP-specific terminology appears to have stabilised in the speciation literature around the term conspecific pollen/sperm precedence in the last few decades; potentially because of its resemblance with

last-male precedence, a term already popular in the competitive fertilisation literature. Katakura (1986) seems to have been the first to coin the term "conspecific sperm precedence" while describing Nakano's (1985) CGP observation in ladybirds. Howard et al. (1998) later extended its application to plants as "conspecific pollen precedence." This terminology has also been tweaked to describe the occurrence of CGP among populations of the same species as conpopulation/contypic sperm precedence (Dixon et al., 2003; Rose et al., 2014). Other descriptive, yet less popular terms to report the occurrence of CGP have included assortative fertilisation (Hewitt et al., 1989), selection against foreign pollen (Hauser et al., 1997), conspecific fertilisation precedence (Williams et al., 1999), conspecific pollen/sperm preference (Miranda et al., 2010; Song et al., 2002), conspecific pollen advantage (Chapman et al., 2005), and postpollination assortative mating (Ostevik et al., 2016). Likewise, other studies have used broader terms that captured selective fertilisation phenomena, including gametic selection (Hornby & Li, 1975), interspecific sperm precedence (Robinson et al., 1994), pollen competition (Baker & Shore, 1995), heterospecific/interspecific pollen competition (Carney et al., 1996; Rieseberg et al., 1995), sperm preemption (Brown & Eady, 2001), assortative mating (Bierne et al., 2002), pollen/sperm precedence (Husband et al., 2002), premating isolation (Willis et al., 2006), nonrandom mating (Carlson et al., 2009), postpollination nonrandom mate selection (Bhattacharya & Baldwin, 2012), and gametophyte competition (Briscoe Runquist et al., 2014).

Besides lacking a standard terminology to refer to CGP, society prejudices around polygamy might have also impacted the early advance of CGP studies. For instance, even though Darwin (1868) opened the possibility for the occurrence of selective fertilisation in animals "if the female before each birth received several males", he immediately discarded it due to his assumption of the prevalence of monogamy in terrestrial animals. Curiously, Darwin seems to have been aware of the occurrence of extra-pair copulations in females but opted to omit it to avoid embarrassment with his family and the Victorian society (Birkhead, 2010). This might have contributed to shape the research agenda of selective fertilisation for most part of the twentieth century, where studies were primarily conducted in plants, with equivalent studies in animals taking several decades to start being conducted at a steady pace (Figure 1). Similarly, Darwin's dismissal of female agency might have contributed to the perpetuation of gender stereotypes in the study of sexual selection more broadly (Rosenthal & Ryan, 2022). Therefore, male-centred investigations have often preceded female-centred

equivalents (Ah-King, 2022a, 2022b). In the context of studying the mechanisms that produce biases in fertilisation, cryptic female choice was proposed as a mechanism of post-copulatory sexual selection only years after the formulation of sperm competition (Parker, 1970; Thornhill, 1983), even though equivalent sexual selection processes occurring before mating had long been discussed in evolutionary biology literature (Ah-King, 2022a). Today, still, sperm competition studies greatly outnumber cryptic female choice studies (Ah-King, 2022b).

Methodological considerations of detecting CGP

Demonstrating the occurrence of CGP requires conducting competitive crossing experiments, scoring the outcome of fertilisation, and assessing biases in paternity. A traditional approach has consisted in taking an egg donor and offering it equal and simultaneous access to both conspecific and heterospecific pollen/sperm. If more than half of the offspring are scored as purebred, it is often assumed that CGP mechanisms biased the fertilisation towards the conspecific gametes (Figure 2A). However, several factors other than CGP can produce the same paternity pattern. Below, we explore those technical and biological limitations, examine the conditions that a crossing experiment should meet to account for them, and describe the statistical approaches to assess the occurrence of CGP in groups with different fertilisation modes. Specifically, we lay out four rules of thumb for experiments aimed at detecting CGP in plants and animals: i) conspecific and heterospecific gametes must be offered the same chances to compete for fertilisation in competitive crosses, ii) the fertilisation advantage enjoyed by conspecific over heterospecific gametes in competitive crosses should exceed the expectations drawn from observed fertilisation rates in non-competitive crosses, iii) the estimation of the paternity share of competitive crosses must not be conflated with variation in postzygotic phenomena, and iv) the fertilisation advantage of conspecific over heterospecific gametes should be assessed in a statistical model that account for the above and other confounding factors whenever they cannot be experimentally controlled. By delineating this roadmap, we acknowledge that many aspects of sexual reproduction are idiosyncratic to particular groups of organisms, each of them imposing its own limitations and opportunities to experimentally study the occurrence of CGP.

A fair assessment of differences in competitive fertilisation abilities requires either preparing pollen or ejaculate mixtures of known proportions of viable gametes of each type

or carefully controlling the conditions of successive copulation experiments (Figure 2B). The concentration and viability of gametes can be readily estimated using traditional microscopy and staining techniques, cell counters, or impedance flow cytometry (Heidmann et al., 2016; Holman, 2009; Kearns & Inouye, 1993). However, this approach works best in plants and animals with external fertilisation where gametes can be collected and mixed in desired proportions. In animals with internal fertilisation, where the sperm from the first copulation might get partially depleted in the interval between copulations, a proxy to control for the quantity of ejaculates is standardising the duration of each copulation and time window between events as well as conducting experiments in different mating orders (see below). Variation in experimental conditions that might affect the viability of gametes should also be minimised, which might include age and storing conditions of both gametes and parental individuals (Reinhardt et al., 2015). In self-compatible hermaphroditic systems, pollen donors must be emasculated to avoid introducing a competitive advantage toward conspecific gametes. This precaution is also necessary when working with pseudo-incompatible plants where the presence of heterospecific pollen can induce self-fertilisation through the mentor effect (Desrochers & Rieseberg, 1998). Further, the ratio of pollen/sperm with the number of available eggs should be high enough to guarantee the manifestation of competitive interactions and fertilisation to happen, but not too high to increase the risk of polyspermy or any other dosage-dependent adverse reactions (Levitan et al., 2007). The pollen/sperm load sizes should also be consistent across replicates of non-competitive and competitive crosses to keep the intensity of competitive interactions constant (Cruzan & Barrett, 1996).

A careful crossing design can further help distinguish whether the fertilisation success of conspecific gametes increases in response to the presence of heterospecific gametes or not. In most plants and animals with external fertilisation, the baseline fertilisation rate of conspecific and heterospecific gametes can be established through non-competitive crosses and then used to ask whether the paternity share of conspecific gametes in competitive crosses reflects an increase in their fertilisation rate (Figure 2C). Ideally, both the competitive and non-competitive crosses should use the same set of individuals to minimize variation. In animals with internal fertilisation where last-male sperm precedence occurs, the focus must shift to the fertilisation rate of the second male to copulate with the egg donor (P_2). To distinguish last-male precedence from CGP, it is necessary to either perform competitive crosses within and between species or perform competitive crosses between species alternating the mating order. By comparing P_2 values between those cross categories, it is

possible to assess whether P_2 varies in a direction that reflects an increase in the fertilisation ability of conspecific sperm (see below).

Even though assessing differences in fertilisation rates is the cornerstone to determining the occurrence of CGP, the direct observation of fertilisation is unfeasible in most systems. Instead, traditional experiments rely on proxy measurements of fertilisation success that might conflate CGP with postzygotic isolation (Garlovsky et al., 2024). Whereas fertilisation rate in non-competitive crosses is commonly assessed via measurements of fecundity such as clutch size, hatching success, seed set, and fruit set (Figure 2C), its measurement in competitive crosses relies on determining the paternity share of the offspring (Figure 2D). This might lead to underestimating the fertilisation rate of heterospecific gametes if postzygotic isolation occurs. For instance, endosperm development is commonly arrested in hybrid seeds (Köhler et al., 2021), making seed set a likely biased proxy of fertilisation rate. Since the chances for a postzygotic reproductive barrier to express increases over development, experiments should aim at scoring fertilisation success as early as technically possible. Nonetheless, differences in offspring survival can be statistically accounted for if survival is scored in the offspring of non-competitive crosses at the same developmental stage in which the paternity of competitive crosses is assigned. In groups where the developing offspring display competitive interactions, the offspring of competitive crosses should be reared individually, or an additional experiment should be set up to quantify the magnitude of competition and statistically account for it (Price et al., 2000).

Whereas scoring the offspring of a competitive cross might reveal an excess of purebred over hybrid individuals, that alone does not constitute proof of the occurrence of CGP. The observed paternity ratio is the compounded result of experimental conditions and biological phenomena, of which CGP is only one factor. The formulation of a fertilisation null model allows to disentangle the contribution of CGP to the observed paternity pattern and statistically assess its occurrence (Figure 2E). In absence of CGP, the expected paternity share of purebred offspring might take any value between 0% and 100%, not necessarily the 50% that is commonly assumed. This depends on the initial ratio of viable gametes, the expression of both non-competitive gametic and postzygotic reproductive barriers, the accuracy of the paternity assignment method, and other factors mentioned above. Then, performing non-competitive fertilisation experiments along competitive ones becomes necessary to measure the magnitude of those confounding variables. A system-specific

fertilisation null model that incorporates the relevant sources of bias will ultimately determine the expected fertilisation ratio in absence of CGP. Given proper replication of crossing experiments and adequate sample sizes (e.g., Kleppe et al., 2018), the statistical assessment of differences in fertilisation rates can proceed through different methods depending on the fertilisation mode of the organisms. This can involve either tests that compare two proportions or tests that assess differences among crossing categories. Since these data are usually expressed as fertilisation proportions, variation gets compressed towards the end of the range, which requires either transforming it or using statistical methods that automatically take this into account.

In plants and animals with external fertilisation, the expected paternity ratio can be directly compared with the observed paternity ratio to ask whether there is an excess of purebred offspring that can be ascribed to the occurrence of CGP (Figure 2E, upper panel). These ratios are commonly expressed as the proportion of purebred offspring. In animals with internal fertilisation where last male precedence occurs and it is feasible to distinguish the offspring of different individuals from the same species, the assessment of CGP proceeds by comparing the P_2 values of competitive crosses within and between species (Figure 2E, lower panel). However, the observed P_2 values must first be corrected to account for the confounding variables discussed above (Figure2B-D). If CGP has evolved, the corrected P_2 values of the focal species should be higher in crosses between species than within species. If measuring P_2 in competitive crosses within species is unfeasible, the expected paternity ratio can be used to estimate the magnitude of the P_2 precedence in competitive crosses between species, defined as the difference between the observed and expected P_2. If CGP is present, the magnitude of P_2 precedence should be higher in crosses where a conspecific individual copulated after a heterospecific one than in the alternative copulation order. In systems where premating isolation prevents the mating of a heterospecific individual after a conspecific mating event, the fertilisation rate of the first male to copulate with the egg donor (P_1) can be used instead. However, P_1 and P_2 might provide insights about two different aspects of CGP, namely defensive and offensive competition, respectively (Turissini et al., 2018).

Case studies

Finding an excess of purebred offspring after conducting competitive crosses seems to be a common observation in different groups of plants and animals, suggesting that CGP

might be a widespread phenomenon in nature. Yet, few efforts have been made to survey and synthesise the CGP literature since Howard's (1999) influential review in which he uncovered 10 case studies: Three in plants and seven in animals. Two notable exceptions are Howard's et al. (2009) and Garlovsky's et al. (2024) surveys of PMPZ studies. While Garlovsky et al. (2024) reported 44 case studies of CGP in plants and 35 in animals, Howard et al. (2009) reported 51 case studies of PMPZ in animals without distinguishing whether they constituted a case of CGP or non-competitive gametic isolation. Furthermore, to assess the occurrence of CGP, Garlovsky et al. (2024) relied on proxies that did not necessarily require conducting competitive crosses or assessing paternity. Whereas those studies have led us to appreciate the pervasiveness of PMPZ reproductive barriers in nature and their role in speciation, they lacked a standardised framework to assess the occurrence of CGP. Below, we synthesise the findings of a survey of the contemporary speciation and reproductive biology literature (1968-2025) in which we assessed the experimental evidence for the occurrence of CGP. We focused on studies published in English that conducted competitive fertilisation experiments and scored the paternity of the offspring, seemingly detecting an excess of purebred individuals. Whereas the use of standard PMPZ terminology in bibliographic databases helped us identify many CGP studies, we discovered many more of them by tracing both relevant references within that initial set of articles and citations of those articles.

We uncovered the signature of CGP in 93 case studies: 41 in plants and 52 in animals (Figure 3; Table S2). These studies span 57 years of research, covering a period in which it became customary to study CGP from a speciation perspective (Figure 1). However, not all studies explicitly aimed at assessing the occurrence of CGP; some of them did so incidentally while characterising other reproductive biology aspects in their group of interest, especially in crop species. Overall, 85 unique publications have empirically assessed the occurrence of CGP. A few of them have tested two pairs of taxa at once (Berger & Rybacki, 1992; Heiser et al., 1969; Pereira et al., 2012; Price, 1997; Smith, 1970) or, extraordinarily, 19 pairs of taxa (Turissini et al., 2018). In two instances, competitive fertilisation experiments were performed involving three or four taxa at once (Lepais et al., 2013; Martín-Coello et al., 2009). Also, 14 taxon pairs have been examined in more than one publication. Even though the findings of multiple publications on the same taxon pair are rarely conflicting, the ones that offer mixed evidence underscore the importance of sampling individuals across a species range to capture natural variation in CGP. For instance, whereas a survey in a hybrid zone of

Ipomopsis aggregata and *I. tenuituba* failed to find evidence of the occurrence of CGP (Alarcón & Campbell, 2000), a study that surveyed a hybrid zone with a different demographic dynamic revealed that CGP was polymorphic across the range of this species pair (Aldridge & Campbell, 2006). Together, this wealth of studies reveals some emerging patterns about the study and occurrence of CGP, some of which we explore next.

There are taxonomic biases in the study of CGP (Figure 3A). In plants, competitive fertilisation experiments have only been conducted in 15 orders of angiosperms. Out of them, Asterales and Ericales comprise more than one third of the case studies. Further, there is no evidence of CGP studies conducted in bryophytes and early diverging vascular plants. In animals, the order Diptera accounts for more case studies than 11 orders of cnidarians, molluscs, echinoderms, and vertebrates combined. This pattern is driven by studies conducted in *Drosophila* species (22 out of 23 case studies). Notably, the dearth and shortcomings of case studies in terrestrial vertebrates contrasts with the richness of reproductive biology studies in this group. Whereas two case studies in mice failed to capture the interactions between ejaculates and the reproductive tract of the ova donor by conducting *in vitro* assays (Dean & Nachman, 2009; Martín-Coello et al., 2009), three case studies in frogs have been either reported anecdotally (Bogart, 1980) or further studies have cast doubts on them (Berger & Rybacki, 1992, 1994; Reyer et al., 2003). In birds, the occurrence of CGP has been suggested based on *in vitro* differences in sperm swim velocity and paternity patterns in the wild (Cramer et al., 2016). However, no studies in this group have conducted competitive fertilisation experiments yet. Similarly, while some studies have documented the occurrence of selective fertilisation in lizards and snakes (Friesen et al., 2020), CGP has not been experimentally assessed in reptiles. Even though we restricted our survey to case studies in plants and animals, it is important to note that CGP can also occur in fungi. For instance, competitive fertilisation experiments revealed that stroma of the endophytes *Epichloë typhina* and *E. clarkia* are preferentially fertilised by conspecific spermatia (Treindl & Leuchtmann, 2019).

The known case studies have also suggested that CGP can evolve at shallow evolutionary timescales: More than one fifth of cases occurred within species (Figure 3B). Those intraspecific systems have studied lineages deemed as allopatric populations (Brown & Eady, 2001; Garlovsky et al., 2020; Ludlow & Magurran, 2006; Rose et al., 2014; Wade et al., 1995), ecotypes (Ostevik et al., 2016), cytotypes (Castro et al., 2020), cultivars (Hornby

& Li, 1975), races (Hewitt et al., 1989), or subspecies (Bella et al., 1992; Briscoe Runquist et al., 2014; Dixon et al., 2003; Nagy, 1997). Together, they have captured the evolution of CGP in evolutionary divergence windows that are critical for the study of speciation. On the other end of the evolutionary divergence continuum, the experimental setup of CGP studies has also been harnessed to test the efficacy of competitive gametic interactions in preventing introgression using backcrosses. In *I. aggregata*, conspecific pollen exhibited a competitive advantage over *I. aggregata* x *I. tenuituba* F1 and F2 hybrid pollen (Campbell et al., 2003). This type of experiment has also been conducted in sunfishes, but no biases in fertilisation were detected (Immler et al., 2011). Similarly, CGP has been studied between a hybrid species and one of their parental species in both plants and animals (Berger & Rybacki, 1992; Hauser et al., 1997). In plants, other common experimental setups included assessing taxa that differ in ploidy (Baack, 2005; Husband et al., 2002; Ishizaki et al., 2013; Koutecký et al., 2011; Smith, 1968; Williams et al., 1999) or mating system (Briscoe Runquist et al., 2014; Brock, 2009; Diaz & Macnair, 1999; Figueroa-Castro & Holtsford, 2009; Hauser et al., 1997; Kiang & Hamrick, 1978; Orsucci et al., 2025; Ruane & Donohue, 2008).

In almost three quarters of the case studies, competitive fertilisation experiments were conducted using individuals from both species as egg donors. In these reciprocal crosses, it was twice as common to detect CGP in both species than in just one (Figure 3C). Even though the analyses of these discrete categories suggest that CGP occurs more often as a symmetric reproductive barrier than an asymmetric one, the magnitude of CGP might exhibit large variation between cross directions, whose quantitative assessment is beyond the scope of this review. This apparent asymmetry of CGP seems to occur more commonly in plants than in animals. However, conducting bidirectional crosses in animals with internal fertilisation was sometimes precluded by asymmetries in premating isolation (Castillo & Moyle, 2014; Price, 1997; Turissini et al., 2018). The pooling of the eggs of both species in "mass-mating" experiments also precluded assessing the directionality of CGP at least in one study (Bierne et al., 2002). A limited number of apparently asymmetric case studies in plants where one species is a selfer and the other is an outcrosser suggests that asymmetries in CGP might be associated with differences in mating system. Whereas CGP was only detected in the outcrossing species in four instances (Diaz & Macnair, 1999; Figueroa-Castro & Holtsford, 2009; Kiang & Hamrick, 1978; Orsucci et al., 2025; Ruane & Donohue, 2008), CGP was only detected in the selfing species once (Hauser et al., 1997).

Standard of evidence

Our survey reflects that the number of CGP studies has reached a critical point, allowing for investigations into its mechanisms, evolution, and consequences for speciation. However, the case studies that support the occurrence of CGP are very variable in methodological approaches, and their standard of evidence must be critically assessed before drawing broader conclusions. The ratio of purebred over hybrid offspring obtained through competitive fertilisation experiments is a reliable indicator of the occurrence of CGP as long as key experimental conditions have been carefully controlled or their confounding effects have been accounted for by a fertilisation null model (Figure 2). Below, we assess four aspects of the competitive fertilisation experiments that support each of the case studies of CGP that we identified above. We scored whether the experiments accounted for differences in i) the ratio of viable gametes, ii) non-competitive fertilisation rate or intraspecific competitive ability, and iii) viability of purebred and hybrid offspring. We also scored iv) whether the biases in fertilisation were assessed in a statistical framework. Failing to comply with any of our criteria does not necessarily invalidate a case study since it is still possible that the unaccounted variables did not induce any biases in the assessment of fertilisation ratios. Instead, we want to encourage future studies to use our proposed roadmap and statistical framework to better document and understand CGP across the different lineages of plants and animals.

The most common experimental consideration that was accounted for across CGP studies was to control the initial quantity of gametes or mating order (89%; Figure 4; Table S2). In plants, this was commonly achieved by pooling pollen loads by mass or volume, using a standardised number of anthers of each type, or sequentially rubbing floral structures in reciprocal order. In animals with external fertilisation, mixing aliquots of known concentration was a routine practice. In animals with internal fertilisation, some studies additionally controlled for copulation rate (Wade et al., 1994), number of transferred spermatophores or attachment time (Gregory & Howard, 1994; Howard & Gregory, 1993; Tyler et al., 2013), or minimum copulation time (Chang, 2004; Dixon et al., 2003; Fricke & Arnqvist, 2004; Price, 1997). Nonetheless, few studies went beyond to further control for differences in the viability of gametes (Bhattacharya & Baldwin, 2012; Dean & Nachman, 2009; Husband et al., 2002). Interestingly, one study in sea urchins indirectly achieved this by simultaneously performing both non-competitive and competitive crosses using the same

sperm dilution (Geyer & Palumbi, 2005). Their expected paternity ratio was drawn from differences in the fecundity of non-competitive crosses, which were the compounded result of differences in gamete viability and non-competitive fertilisation rate.

Over one third of the experiments conducted either non-competitive crosses to separately estimate the fertilisation rate of each species or competitive crosses within species to determine intraspecific competitive ability. Whereas this was a common practice in studies conducted in insects (76%), it was scarcely part of the experiments conducted in plants (8%). However, studies in insects that measured P_2 within species only occasionally incorporated this measurement into a fertilisation null model (29%). A study that failed to find evidence of CGP in tree frogs illustrates the importance of measuring non-competitive fertilisation rates (Sherman et al., 2010); their experiments revealed that differences in non-competitive fertilisation alone explained observed biases in competitive fertilisation experiments. Crucially, non-competitive crosses also provide information about whether heterospecific crosses can be successful at all, allowing to differentiate when the apparent occurrence of CGP is just the reflection of non-competitive gametic isolation (e.g., Noriyuki et al., 2012). Most of the studies that estimated differences in non-competitive fertilisation rate or intraspecific competitive ability also accounted for differences in the initial quantity of gametes or alternated mating orders (92%).

Competitive fertilisation experiments least frequently measured differences in survival between purebred and hybrid offspring (27%). Nonetheless, the ones that did also tended to account for additional confounding factors in a statistical framework. Studies used survival measurements to either rule out any differences or account for them in fertilisation null models. Depending on the paternity assignment method, the offspring were scored at different developmental stages, ranging from 1.5-day larvae to sexually matured individuals. However, the developmental stages at which survival was estimated and paternity was assigned did not always match. Ascertainment biases in paternity assignment methods were also rarely assessed. The most common techniques were scoring morphological traits (body colouration, size, and genitalia shape) and revealing allozyme and genetic markers (amplicons, microsatellites, AFLP, RFLP, SCAR, SSR). Other studies assessed paternity based on differences in karyotype (Bella et al., 1992; Bogart, 1980; Hewitt et al., 1989), nuclear DNA content (Baack, 2005; Baldwin & Husband, 2011; Castro et al., 2020; Husband et al., 2002; Koutecký et al., 2011), hatchability (Brown & Eady, 2001; Garlovsky et al.,

513 2020; Rugman-Jones & Eady, 2007), offspring fertility (Smith, 1970; Wade et al., 1994), and
514 seed abortion (Orsucci et al., 2025). In two in vitro experiments in mice, it was feasible to
515 score the paternity of recently fertilised eggs (Dean & Nachman, 2009; Martín-Coello et al.,
516 2009).

517 The occurrence of CGP was assessed in a statistical framework in half of the studies.
518 Depending on the fertilisation mode, analyses aimed at either comparing the observed and
519 expected proportion of purebred offspring or testing whether variation in P_2 was affected by
520 species or mating order. In plants and animals with external fertilisation, statistical tests
521 included chi-squared test, G-test, t-test, binomial test, binomial 95% confidence intervals,
522 Hardy-Weinberg exact test, one sample proportion test, repeated measures ANOVA,
523 Duncan's new multiple range test, and parametrised maximum likelihood models. In animals
524 with internal fertilisation, statistical tests included generalised linear model, Mann-Whitney
525 U-test, logistic regression, G-test, generalised linear mixed model, analysis of deviance,
526 three-way nested ANOVA, and Fisher's exact test. Even though many experiments conducted
527 multiple replicates of competitive crosses, they often pooled their offspring or analysed them
528 without accounting for pseudo-replication due to the repeated use of the same egg or sperm
529 donors. Curiously, studies that assessed CGP along with other reproductive barriers in the
530 same system often restricted their analyses of CGP data to estimate standard metrics of
531 absolute and relative contribution to reproductive isolation (e.g., Sobel & Chen, 2014), even
532 though their data would be suitable to formulate fertilisation null models and statistically
533 assess the occurrence of CGP.

534 Perspectives for the study of CGP

535 Gametic interactions are ubiquitous in plants and animals as well as in other groups of
536 sexually reproducing eukaryotes, making CGP a potentially common reproductive barrier in
537 nature. Proposed over 260 years ago as a process that can limit hybridization (Kölreuter,
538 1763, 1766), CGP only started to make its way into mainstream speciation research a few
539 decades ago (Figure 1). Today, the exploration of a limited number of plant and animal
540 clades have uncovered its apparent occurrence in 93 taxon pairs (Figure 3). Despite this
541 growing interest in the study of CGP, the field does not yet seem ready for drawing
542 quantitative comparative inferences because published results often failed to account for
543 confounding factors (Figure 4). Above, we delineated an experimental roadmap to assess the

occurrence of CGP (Figure 2), which can be harnessed to both reanalyse published datasets and design new CGP investigations. Along with the adoption of a standardised framework to measure and report reproductive isolation observations (Sobel & Chen, 2014; Walker et al., 2026), this roadmap can help CGP catch up with other areas of speciation research. As those developments take place, we highlight other research avenues that can also help us understand how this fundamental reproductive barrier has contributed to the evolution of plants and animals.

Studying the richness of competitive gametic interactions can offer fertile ground to understand the mechanistic basis of CGP. Depending on the group, they encompass diverse interactions among gametophytes, sperm cells, eggs, ejaculates, and reproductive tissues and their products, each one potentially offering opportunities for the emergence of reproductive advantages to conspecific gametes. Even though studies of CGP mechanisms enjoyed a head start in plants (Gärtner, 1849), we know little about the molecular underpinning of the accelerated growth or differential arrestment of pollen tubes in the context of interspecific competitive fertilisation, to name just a few of the potential mechanisms of CGP in flowering plants. Although those interactions might have polygenic architectures (Fishman et al., 2008), recent work has started to elucidate key players of pollen-pistil interactions that mediate pollen tube recognition and differential growth (Baraniecka et al., 2024; Bhattacharya & Baldwin, 2012; Guo et al., 2019). Studies on broadcast marine invertebrates have also offered insights on gametic lock-and-key systems that confer high specificity to fertilisation (Palumbi, 1999; Vacquier, 1998); however, it is unclear how they might provide a competitive advantage to conspecific spermatozoa beyond conferring non-competitive gametic isolation. We know less about this process in fishes and other externally reproducing species, where differences in sperm swimming speed and its modulation by ovarian fluids have been suggested as mechanisms of CGP (Devigili et al., 2018; Liljedal et al., 2008; Yeates et al., 2013). In contrast, more effort has been put into understanding the mechanisms of CGP in animals with internal fertilisation, mostly in *Drosophila* species. These studies have revealed ways in which heterospecific sperm is differentially inactivated or ejected or its performance affected by the female reproductive tract (Manier, Belote, et al., 2013; Manier, Lüpold, et al., 2013; Price, 1997; Price et al., 2000; Rugman-Jones & Eady, 2007; Tyler et al., 2013). Still, their genetic bases remain poorly understood.

Considering that competitive gametic interactions can also mediate inbreeding avoidance (Bretman et al., 2009; Dorsey & Rosenthal, 2023; Firman & Simmons, 2015; Gasparini & Pilastro, 2011; Mack et al., 2002; Stockley, 1999; Zeh & Zeh, 1996), an intriguing question is to what extent CGP shares its molecular machinery with inbreeding avoidance mechanisms, and how this can facilitate or constrain its evolution under different demographic scenarios. Even though sexual selection has also been proposed as an evolutionary driver of competitive gametic interactions (Birkhead et al., 1993; Birkhead & Pizzari, 2002; Eady, 2001), few studies have explored the mechanistic links between sexual selection and CGP (Castillo & Moyle, 2014). Compared with the array of traits affecting prepollination and premating phenomena, CGP might offer restricted physiological targets of selection that might impose lower costs to its evolution via reinforcement (Garlovsky et al., 2024; Long et al., 2026), whose plausibility has been explored in theoretical models (Lorch & Servedio, 2007; Marshall et al., 2002; Rushworth et al., 2022). Nonetheless, empirical evidence of the evolution of CGP by reinforcement is still mixed (Castillo & Moyle, 2019; Turissini et al., 2018). In the context of the evolution of other reproductive barriers, a single comparative study has been carried out, which suggests that CGP can evolve faster than hybrid inviability but slower than premating isolation in *Drosophila* (Turissini et al., 2018). Notably, our empirical insights into the evolution of CGP mostly come from studies conducted in animals.

The development of novel methodological approaches to study competitive fertilisation phenomena can provide tools to overcome some of the current limitations to investigate the occurrence, mechanisms, and evolution of CGP. Except in some groups of echinoderms where the fertilisation membrane lifts off, direct observations of syngamy are unfeasible in most plants and animals (Barresi & Gilbert, 2020); even in those few exceptions, distinguishing the identity of the fertilising spermatozoa is beyond reach. However, some studies have either genetically marked pollen or harnessed differences in nuclear DNA content to assign species identity to growing pollen tubes (Swanson et al., 2016; Williams et al., 1999), genetically marked spermatozoa to assess differences in sperm use patterns (Manier, Belote, et al., 2013; Manier, Lüpold, et al., 2013), or used dyes to stain mitochondria or nucleic acids of spermatozoa and score paternity before karyogamy (Dean & Nachman, 2009; Martín-Coello et al., 2009). Similarly, future studies in insects could tap rhodamine B fluorescence staining of seminal fluids through sugar-feeding to design competitive fertilisation experiments in a cost-effective manner (Blanco et al., 2006; Johnson

et al., 2017). Plant species with free-swimming sperm cells, some of which have advanced molecular genetic resources (Bowman et al., 2022; Zhang et al., 2026), are also primed for conducting these types of experiments and testing for the occurrence of CGP outside flowering plants. Lastly, we highlight two additional research avenues with potential implications for biodiversity conservation and biosecurity. One, considering that environmental conditions can affect the expression of CGP (Ruane & Donohue, 2008) and rising temperatures risk disrupting several aspects of fertilisation in plants and animals (Denney et al., 2026; Snook et al., 2026), it is important to assess the effect of different environmental stressors on competitive gametic interactions to forecast how projected global change might impact the coexistence of species, especially those for which CGP represents an important reproductive barrier. Two, the identification of the genetic basis of CGP in plants could contribute to the engineering of transgenic crops with less competitive pollen than that of wild relatives and thus minimising the risk of introgressing transgenes into natural populations (Pons et al., 2015; Song et al., 2002).

Figures

Figure 1. Accumulation of case studies of conspecific gamete precedence from 1968 to 2025. Each case study represents a unique taxon pair in which competitive fertilisation experiments apparently support the occurrence of CGP. There are 93 known case studies of CGP: 41 in plants, 34 in insects, 10 in vertebrates, and 8 in marine invertebrates. See Table S2 for a list of these case studies.

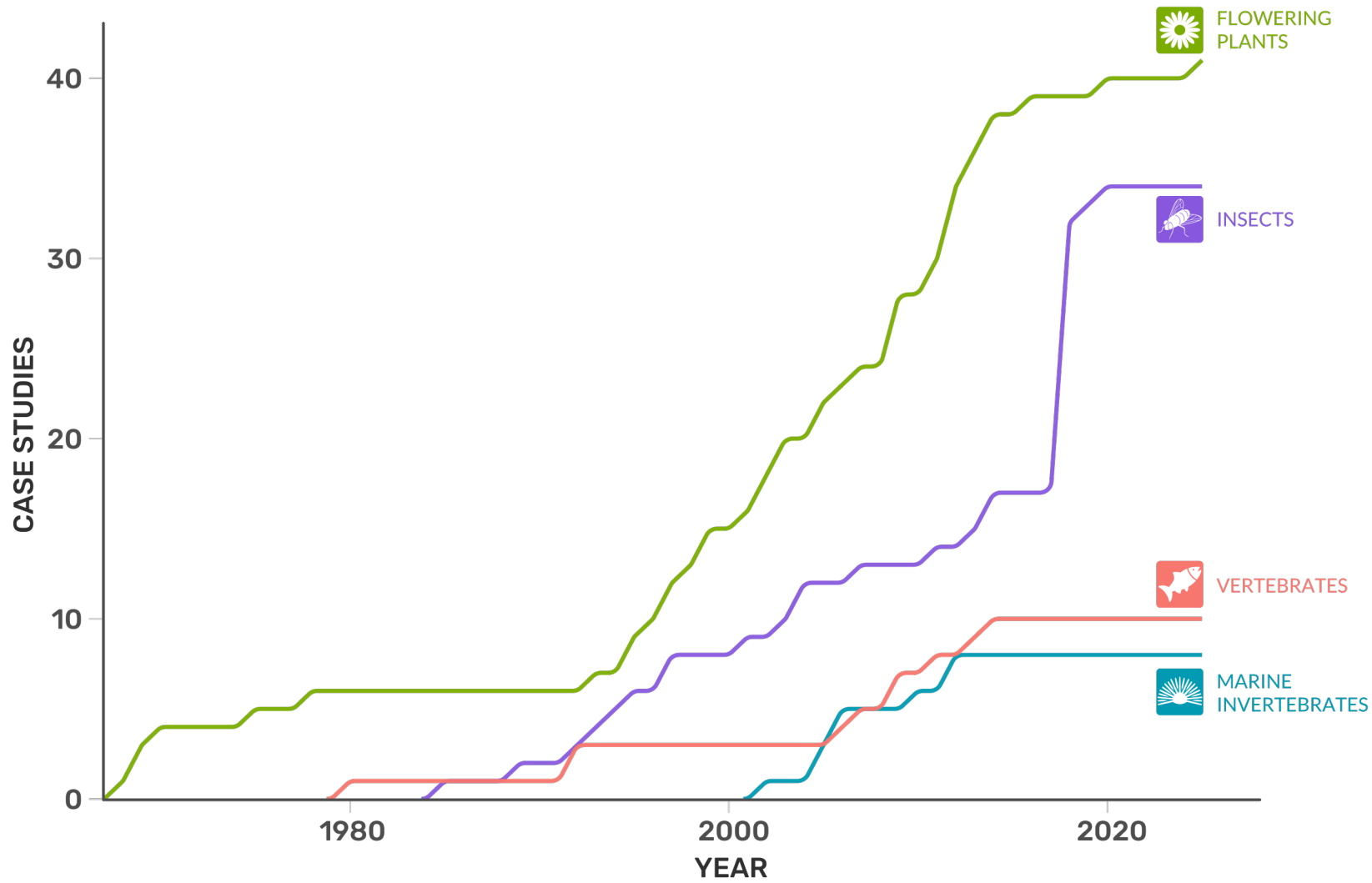
Figure 2. The design of experiments seeking to demonstrate the occurrence of conspecific gamete precedence. A. Scoring an excess of purebred over hybrid offspring after performing competitive crosses is commonly taken as evidence of the occurrence of CGP. Depending on the group, this entails performing hand pollinations, mixing spawned gametes, or setting up successive copulations. However, 50% purebred offspring in competitive crosses does not constitute a default null model against which to test for the occurrence of CGP. **B.** Experimental conditions must guarantee equal fertilisation opportunities to both types of gametes. Differences in the quantity or viability of pollen/sperm, or the duration and interval between copulations, can offer an effective numerical advantage to one type of gamete over the other and can thereby confound inferences on CGP. **C.** The fertilisation rate of conspecific and heterospecific gametes in non-competitive crosses may be different and it should be disentangled from estimation of CGP. For CGP to occur, conspecific gametes should enjoy a fertilisation advantage that is not predicted by the outcome of non-competitive gametic isolation. **D.** Paternity assignment approaches must ensure that their observations unambiguously reflect the outcome of fertilisation rather than the action of postzygotic isolation. Differences in survival by the time that the offspring is scored for paternity can increase the representation of one type of offspring over the other. Moreover, paternity assignment methods themselves might introduce additional biases if diagnostic traits to differentiate purebred from hybrid offspring are ambiguous or if it preferentially amplifies a diagnostic allele over the other. **E.** The statistical framework to assess the occurrence of CGP relies on building a system-specific multiplicative fertilisation null model that accounts for all the factors that can bias the observed paternity ratio in absence of CGP. In plants and animals with external fertilisation, the expected paternity ratio is directly compared with the observed paternity ratio to determine whether there is an excess of purebred offspring. In animals with internal fertilisation, the expected paternity ratio is used to either correct the P2 values of competitive

crosses within and between species or measure the magnitude of P2 precedence in competitive crosses that alternate the copulation order between species. The occurrence of CGP is then assessed by comparing either the corrected P2 values or the magnitude of P2 precedence among experimental setups. The black icons indicate either conspecific individuals or purebred offspring, the orange icons indicate either heterospecific individuals or hybrid offspring. The flower, sea urchin, and fly icons represent plants, animals with external fertilisation, and animals with internal fertilisation, respectively.

Figure 3. Patterns of reported conspecific gamete precedence case studies. A. Taxonomic breadth of case studies in plants and animals. The outermost circle groups case studies by order and the next concentric circle further groups them by higher taxonomic categories, corresponding to phylum in animals. In animals, the innermost circle groups case studies based on their fertilisation mode. The size of the circles and slices is proportional to the number of case studies. **B.** Distribution of case studies based on whether the assessed taxa belonged to the same species (intraspecific) or different species (interspecific). They could also involve crosses between hybrid individuals and individuals from one of the parental lineages (backcross). **C.** Distribution of case studies based on whether CGP was assessed in both species. In the cases where it was, it is indicated whether CGP was detected in both species or in just one species. For a taxon pair in which more than one study has been conducted, results offer mixed evidence.

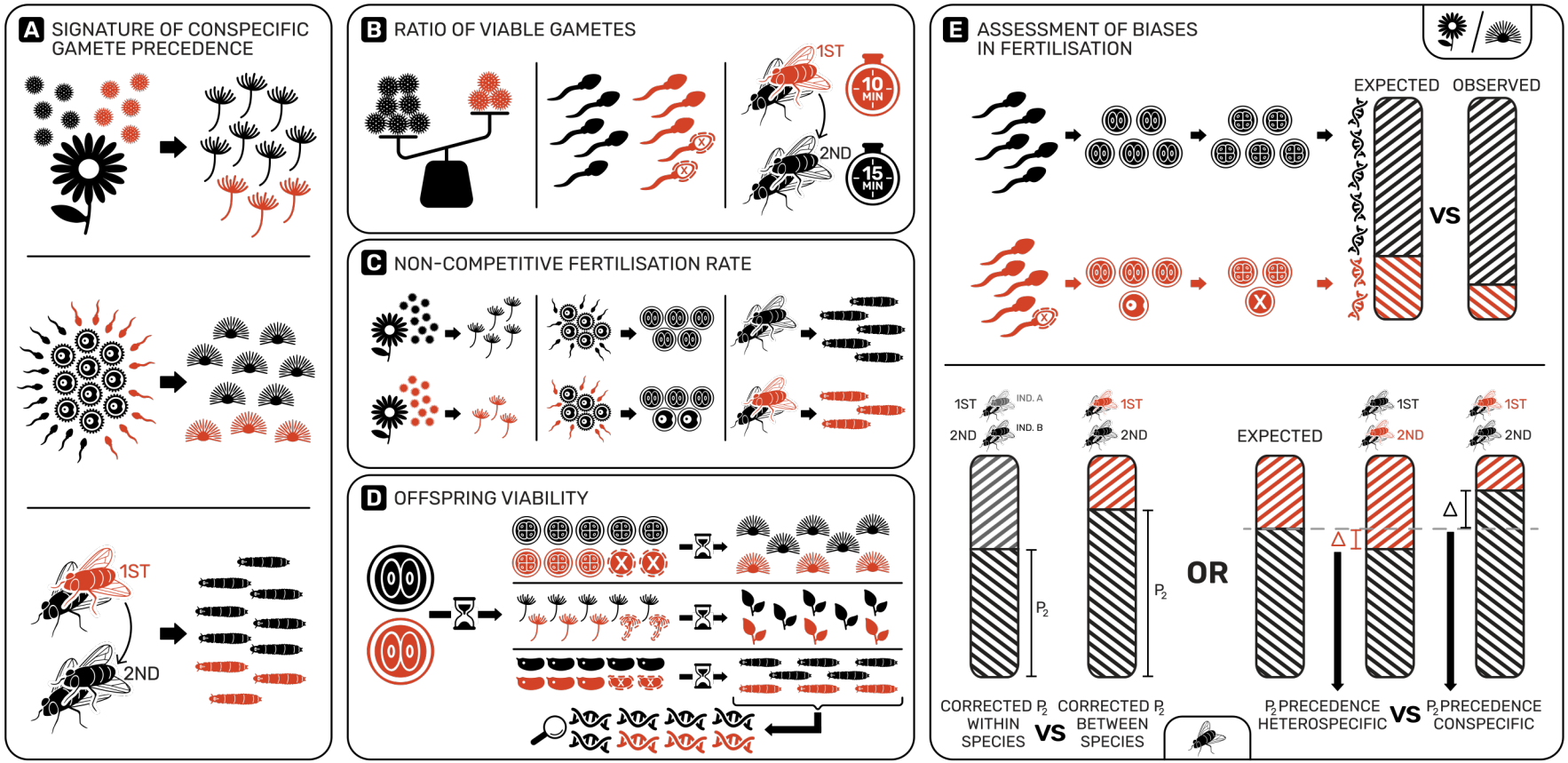
Figure 4. Assessment of the experimental design of case studies supporting the occurrence of conspecific gamete precedence. The left oval contains case studies that controlled for the ratio of viable gametes to some extent, the right oval contains case studies that accounted for differences in non-competitive fertilisation rate or intraspecific competitive ability, and the central oval contains case studies that accounted for differences in survival of the offspring of competitive crosses. The white space in the outer box contains case studies that did not account for any of those experimental conditions. The colour of the icons indicates whether the assessment of biases in fertilisation was conducted in a statistical framework (black) or not (grey).

682 **Figure 1**



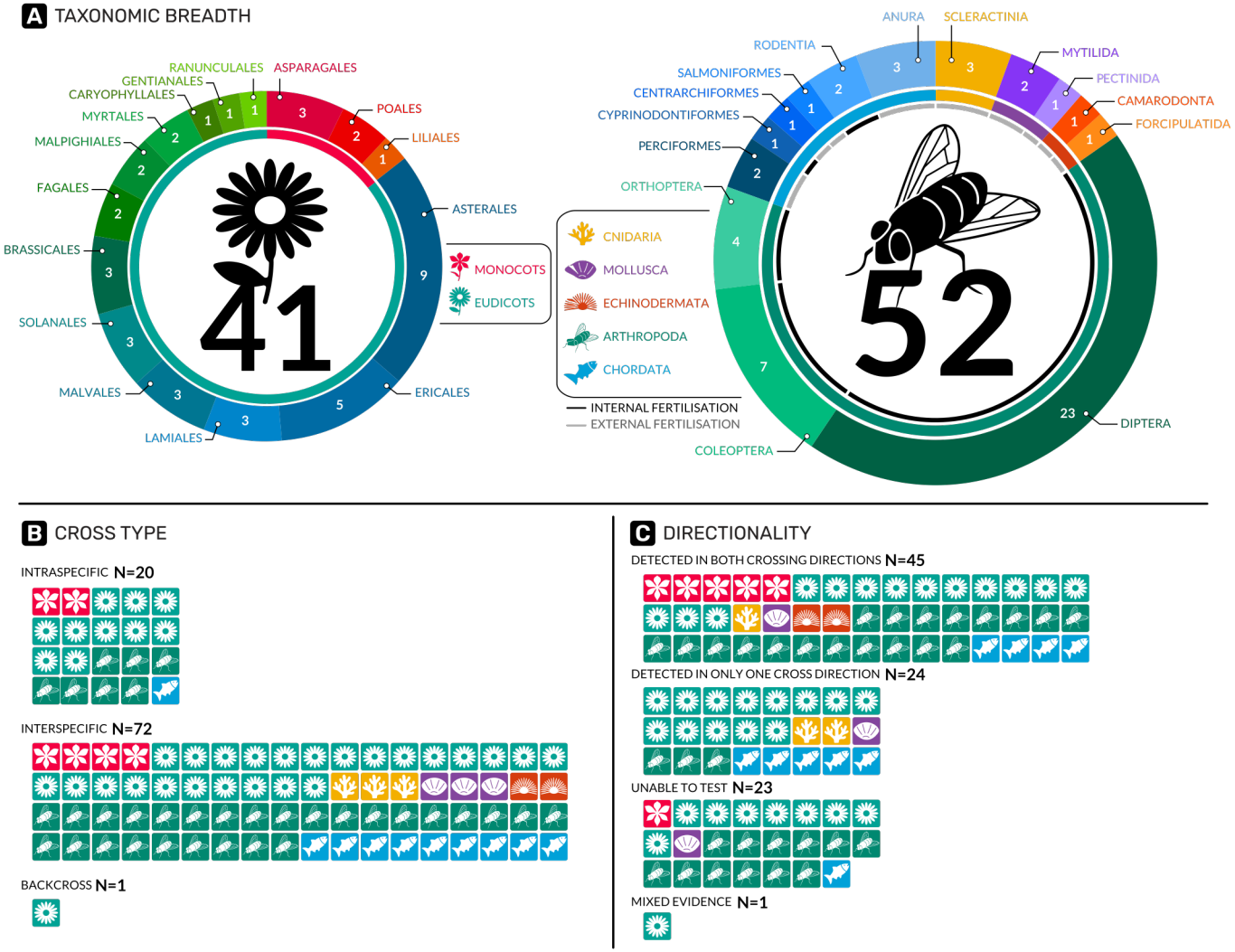
683

684 **Figure 2**



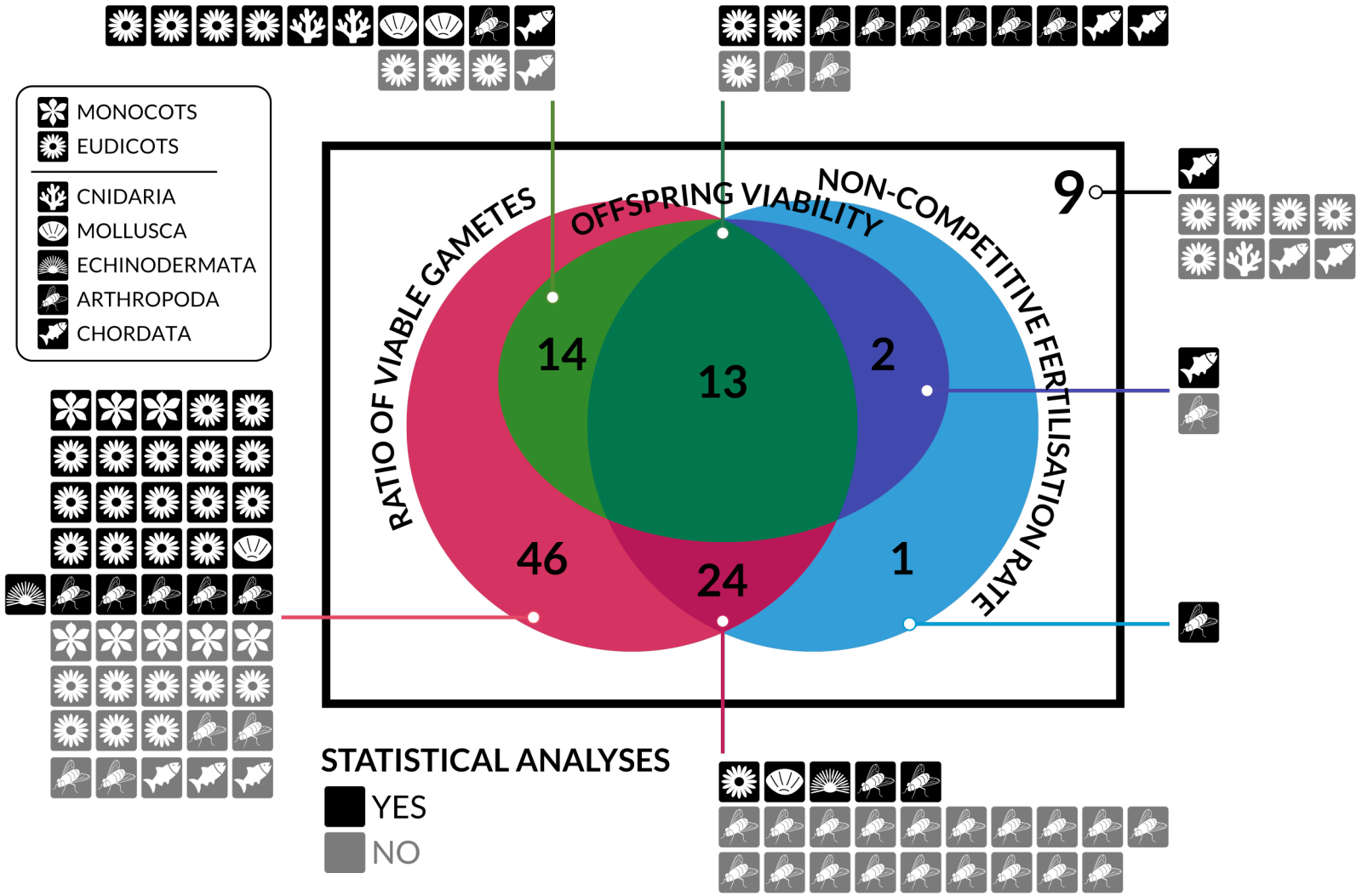
685

686 **Figure 3**



687

688 **Figure 4**



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Data availability

All data is contained in the manuscript.

Author contributions

H.A.C., D.O.B, and J.E. conceptualised the project. H.A.C. conducted data curation. H.A.C. wrote the manuscript with contributions from D.O.B, J.E., R.R.S, and L.R.

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Conflict of interests:

The authors declare no conflict of interest.

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Supplementary Material

Conspecific Gamete Precedence in Speciation:

History, Evidence, and a Research Roadmap

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Table S1. Competitive pollination experiments conducted by Joseph Gottlieb Kölreuter. Kölreuter used different species of *Nicotiana* (*N. rustica*, *N. paniculata*, *N. perennis*, *N. glutinosa*, and *N. major*) and varieties of *Hyoscyamus* (var. *Sibir*. and *aur. cret. mai*.) and *Mirabilis jalapa* (var. *fl. rubr*. and *fl. flav*.). These experiments were diverse in nature. They could 1) cross different varieties of the same species, different species of the same genus, or different species from different genera, 2) include an egg donor that was either purebred or hybrid, 3) mix pollen from either two or three species or varieties in different proportions, and 4) include or exclude pollen from the same species or variety as the egg donor. In addition to these experiments, Kölreuter also conducted non-competitive pollination experiments with most of the species and varieties involved in the competitive crosses, denoted here by an asterisk. Note that the name of species and varieties are reported as per Kölreuter (1763, 1764) and they may have changed since then.

Egg donor	Pollen mixture	Fertilisation outcome	Reference
N. rustica x *N. paniculata* hybrid	*N. paniculata** + *N. perennis*	5 plants did not look like *N. perennis*	Kölreuter (1763), exp. V, p. 20
N. rustica x *N. paniculata* hybrid	*N. rustica** + *N. paniculata** + *N. perennis*	1 plant did not look like *N. rustica* or *N. perennis*	Kölreuter (1763), exp. VI, p. 20
N. rustica	*N. paniculata** + *N. perennis**	5 plants did not look like *N. perennis*	Kölreuter (1763), exp. X, p. 24-25
N. paniculata	*N. rustica** + *N. perennis**	2 plants did not look like *N. perennis*	Kölreuter (1763), exp. XI, p. 25
N. paniculata	*N. paniculata* + *N. perennis**	6 plants looked like *N. paniculata*	Kölreuter (1763), exp. XII, p. 25

N. paniculata	*N. paniculata* + *N. rustica** + *N. perennis**	15 plants looked like *N. paniculata*	Kölreuter (1763), exp. XIII, p. 25-26
N. perennis	*N. perennis* + *N. paniculata**	10 plants looked like *N. perennis*	Kölreuter (1763), exp. XIV, p. 26
N. perennis	*N. perennis* + *N. paniculata** + *N. rustica**	8 plants looked like *N. perennis*	Kölreuter (1763), exp. XV, p. 26
N. perennis	*N. perennis* (little) + *N. glutinosa** (much more)	2 plants looked like *N. perennis*	Kölreuter (1764), exp. XIV, p. 60
N. glutinosa	*N. glutinosa* (little) + *N. paniculata** (much more)	>1 plant looked like *N. glutinosa*	Kölreuter (1764), exp. XV, p. 60
N. glutinosa	*N. glutinosa* + *N. major**	4 plants looked like *N. glutinosa*	Kölreuter (1764), exp. XVI, p. 61
N. rustica	*N. rustica* + *N. paniculata** + *N. perennis**	6 plants looked like *N. rustica*	Kölreuter (1764), exp. XVII, p. 61
N. rustica	*N. rustica* + *N. perennis**	4 plants looked like *N. rustica*	Kölreuter (1764), exp. XVIII, p. 61
N. rustica	*N. rustica* + *Hyoscyamus Sibir*.	>1 plant looked like *N. rustica*	Kölreuter (1764), exp. XIX, p. 62
N. rustica	*N. rustica* + *Hyoscyamus aur. cret. mai.*	≥1 plant looked like *N. rustica*	Kölreuter (1764), exp. XX, p. 62

N. rustica	*N. rustica* (little) + *N. paniculata* (much more)	6 plants looked like normal *N. rustica* x *N. paniculata* hybrids	Kölreuter (1764), exp. XXI, p. 62
N. rustica x *N. paniculata* hybrid	*N. rustica** + *N. paniculata** + *N. perennis*	1 plant looked like *N. rustica*	Kölreuter (1764), exp. XXII, p. 62-65
M. jalapa fl. flav.	*M. jalapa fl. flav.* + *M. jalapa fl. rubr.** (3:2 ratio)	1 plant looked like *M. jalapa fl. flav*.	Kölreuter (1764), exp. XLIX, p. 127
M. jalapa fl. flav.	*M. jalapa fl. flav.* + *M. jalapa fl. rubr.** (5:5 ratio)	1 plant looked like *M. jalapa fl. flav.*	Kölreuter (1764), exp. XLIX, p. 127

Table S2. Case studies of conspecific gamete precedence (1968-2025). Each case study represents a unique combination of taxa, which might have been examined in more than one publication. The case studies are grouped by taxonomic group and sorted by the year of discovery. Comparison type (Comp.) indicates the cross type, which could involve taxa from the same (Intra.) or different (Inter.) species or backcrosses (Back.). For the studies that conducted experiments in both crossing directions, directionality (Dir.) indicates whether the occurrence of CGP was detected in only one or both directions.

Taxa	Higher classification (Order)	Comp.	Citation	Dir.	Ratio of viable gametes	Non-competitive fertilisation rate	Offspring viability	Statistical testing
Iris fulva & *I. hexagona*	Monocots (Asparagales)	Inter.	Arnold et al., 1993	Both	Pollen ratio	Not controlled	Not controlled	NA (lack of variation)
			Carney et al., 1994	Both	Pollen ratio	Not controlled	Not controlled	Goodness of fit G test
			Carney et al., 1996	Both	Pollen ratio	Not controlled	Not controlled	None
Iris fulva & *I. brevicaulis*	Monocots (Asparagales)	Inter.	Emms et al., 1996	Both	Pollen ratio	Not controlled	Not controlled	Chi-squared test
Oryza rufipogon & *O. sativa*	Monocots (Poales)	Inter.	Song et al., 2002	NA	Pollen ratio	Not controlled	Not controlled	None
Sorghum bicolor	Monocots (Poales)	Intra.	Muraya et al., 2011	Both	Pollen ratio	Not controlled	Not controlled	Chi-squared test
Trillium camschatcense & *T. tschonoskii*	Monocots (Liliales)	Inter.	Ishizaki et al., 2013	Both	Pollen ratio	Not controlled	Not controlled	None
Gladiolus communis	Monocots (Asparagales)	Intra.	Castro et al., 2020	Both	Pollen ratio	Not controlled	Not controlled	None

Haplopappus validus	Eudicots (Asterales)	Intra.	Smith, 1968	NA	Not controlled	Not controlled	Not controlled	None
			Smith, 1970	Both	Not controlled	Not controlled	Not controlled	None
Helianthus mollis & *H. occidentalis*	Eudicots (Asterales)	Inter.	Heiser et al., 1969	NA	Not controlled	Not controlled	Not controlled	None
Helianthus grosseserratus & H. mollis	Eudicots (Asterales)	Inter.	Heiser et al., 1969	NA	Not controlled	Not controlled	Not controlled	None
Haplopappus validus & *H. divaricatus*	Eudicots (Asterales)	Inter.	Smith, 1970	Both	Not controlled	Not controlled	Not controlled	None
Lycopersicon esculentum	Eudicots (Solanales)	Intra.	Hornby & Li, 1975	NA	Pollen ratio	Not controlled	Not controlled	Duncan's new multiple range test
Mimulus guttatus & *M. nasutus*	Eudicots (Lamiales)	Inter.	Kiang & Hamrick, 1978	One	Pollen ratio	Not controlled	Not controlled	Unclear
			Diaz & Macnair, 1999	One	Pollen ratio	Not controlled	Not controlled	None
Turnera ulmifolia	Eudicots (Malpighiales)	Intra.	Baker & Shore, 1995	Both	Pollen ratio	Not controlled	Accounted	Goodness of fit G test
Helianthus annuus & *H. petiolaris*	Eudicots (Asterales)	Inter.	Rieseberg et al., 1995	Both	Pollen ratio	Not controlled	Not controlled	Chi-squared test, arcsine-square root transformation
Brassica napus & *B. campestris*	Eudicots (Brassicales)	Inter.	Hauser et al., 1997	One	Pollen ratio	Not controlled	Accounted	Custom maximum likelihood model

Gilia capitata	Eudicots (Ericales)	Intra.	Nagy, 1997	NA	Pollen ratio	Not controlled	Not controlled	None
Piriqueta caroliniana & *P. viridis*	Eudicots (Malpighiales)	Inter.	Wang & Cruzan, 1998	One	Pollen ratio	Not controlled	Not controlled	Goodness of fit G test
Hibiscus moscheutos & *H. laevis*	Eudicots (Malvales)	Inter.	Klips, 1999	Both	Pollen ratio	Accounted	Not controlled	Chi-squared test
Betula occidentalis & *B. papyrifera*	Eudicots (Fagales)	Inter.	Williams et al., 1999	Both	Pollen ratio	Accounted	Accounted	G test, arcsine transformation
Ipomopsis aggregata & *I. arizonica*	Eudicots (Ericales)	Inter.	Wolf et al., 2001	One	Pollen ratio	Not controlled	Not controlled	One-sample t test
Chamerion angustifolium	Eudicots (Myrtales)	Intra.	Husband et al., 2002	One	Pollen ratio & viability	Not controlled	Accounted	None
			Baldwin & Husband, 2011	Both	Pollen ratio	Accounted	Accounted	G goodness-of-fit test with Williams' correction
Ipomopsis aggregata & I. *aggregata* x *I. tenuituba* hybrids	Eudicots (Ericales)	Back.	Campbell et al., 2003	NA	Pollen ratio	Not controlled	Not controlled	One-sample t test
Mimulus lewisii & *M. cardinalis*	Eudicots (Lamiales)	Inter.	Ramsey et al., 2003	One	Pollen ratio	Not controlled	Not controlled	Chi-squared test
Ranunculus adoneus	Eudicots (Ranunculales)	Intra.	Baack, 2005	Both	Pollen ratio	Not controlled	Not controlled	None
Senecio chrysanthemifolius & *S. aethnensis*	Eudicots (Asterales)	Inter.	Chapman et al., 2005	One	Pollen ratio	Not controlled	Not controlled	Chi-squared test, arcsine-square root transformation

Ipomopsis aggregata & *I. tenuituba*	Eudicots (Ericales)	Inter.	Aldridge & Campbell, 2006	One	Pollen ratio	Not controlled	Not controlled	One-sample t test
Phlox drummondii & P. cuspidata	Eudicots (Ericales)	Inter.	Ruane & Donohue, 2008	One	Pollen ratio	Not controlled	Accounted	None
Taraxacum ceratophorum & *T. officinale*	Eudicots (Asterales)	Inter.	Brock, 2009	NA	Pollen ratio	Not controlled	Not controlled	None
Arabidopsis thaliana	Eudicots (Brassicales)	Intra.	Carlson et al., 2009	One	Pollen ratio	Not controlled	Not controlled	Chi-squared test
			Swanson et al., 2016	NA	Pollen ratio	Accounted	Accounted	None
Nicotiana longiflora & *N. plumbaginifolia*	Eudicots (Solanales)	Inter.	Figueroa-Castro & Holtsford, 2009	One	Pollen ratio	Not controlled	Not controlled	Chi-squared test
Silene latifolia & *S. dioica*	Eudicots (Caryophyllales)	Inter.	Rahmé et al., 2009	One	Pollen ratio	Not controlled	Not controlled	Binomial 95% confidence intervals
			Montgomery et al., 2010	One	Pollen ratio	Not controlled	Accounted	Hauser et al. (1997) maximum likelihood model
Centaurea pseudophrygia & *C. jacea*	Eudicots (Asterales)	Inter.	Koutecký et al., 2010	Both	Pollen ratio	Not controlled	Accounted	Chi-squared test
Nicotiana attenuata	Eudicots (Solanales)	Intra.	Bhattacharya & Baldwin, 2012	Both	Pollen ratio & viability	Not controlled	Not controlled	Chi-squared test, log10 transformation

Rhinanthus angustifolius & *R. minor*	Eudicots (Lamiales)	Inter.	Natalis & Wesselingh, 2012	Both	Pollen ratio	Not controlled	Not controlled	One sample proportion test with continuity correction
Gossypium barbadense & *G. hirsutum*	Eudicots (Malvales)	Inter.	Pereira et al., 2012	NA	Pollen ratio	Not controlled	Not controlled	Chi-squared goodness-of-fit test
Gossypium mustelinum & *G. hirsutum*	Eudicots (Malvales)	Inter.	Pereira et al., 2012	NA	Pollen ratio	Not controlled	Not controlled	Chi-squared goodness-of-fit test
Quercus robur, *Q. petraea*, *Q. pubescens* & *Q. pyrenaica*	Eudicots (Fagales)	Inter.	Lepais et al., 2013	Both	Pollen ratio	Not controlled	Not controlled	None
Clarkia xantiana	Eudicots (Myrtales)	Intra.	Briscoe Runquist et al., 2014	Both	Pollen ratio	Not controlled	Not controlled	None
Centaurium littorale & *C. erythraea*	Eudicots (Gentianales)	Inter.	Brys et al., 2014	One	Pollen ratio	Not controlled	Not controlled	None
Helianthus petiolaris	Eudicots (Asterales)	Intra.	Ostevik et al., 2016	Both	Pollen ratio	Not controlled	Not controlled	None
Capsella grandiflora & *C. rubella*	Eudicots (Brassicales)	Inter.	Orsucci et al., 2025	One	Pollen ratio	Not controlled	Accounted	None
Acropora millepora & *A. pulchra*	Cnidaria (Scleractinia)	Inter.	Willis et al., 2006	Both	Not controlled	Not controlled	Not controlled	NA (lack of variation)
Acropora palmata & *A. cervicornis*	Cnidaria (Scleractinia)	Inter.	Fogarty, Vollmer, et al., 2012	One	Sperm concentration	Not controlled	Accounted	Chi-squared test

Montastraea annularis & *M. franksi*	Cnidaria (Scleractinia)	Inter.	Fogarty, Lowenberg, et al., 2012	One	Sperm concentration	Not controlled	Accounted	Paired t-test
Mytilus edulis & *M. galloprovincialis*	Mollusca (Mytilida)	Inter.	Bierne et al., 2002	NA	Sperm concentration	Not controlled	Not controlled	Hardy-Weingberg exact test
Chlamys farreri & *Patinopecten yessoensis*	Mollusca (Pectinida)	Inter.	Lü et al., 2006	Both	Sperm concentration	Not controlled	Accounted	Chi-squared test
Mytilus edulis & *M. trossulus*	Mollusca (Mytilida)	Inter.	Miranda et al., 2010	One	Sperm concentration	Not controlled	Accounted	G test
Echinometra oblonga & *E.* sp. C	Echinodermata (Camarodonta)	Inter.	Geyer & Palumbi, 2005	Both	Sperm concentration & viability	Accounted	Not controlled	Two-tailed bionmial test, 95% CI, arcsine transformation
Asterias forbesi & *A. rubens*	Echinodermata (Forcipulatida)	Inter.	Harper & Hart, 2005	Both	Sperm viability	Not controlled	Not controlled	G test for goodness of fit, Williams correction
			Klibansky & McCartney, 2014	NA	Sperm concentration	Accounted	Not controlled	G test for goodness of fit, Williams correction, two-tailed exact binomial test
Henosepilachna vigintioctomaculata & *H. pustulosa*	Arthropoda (Coleoptera)	Inter.	Nakano, 1985	Both	Mating order	Not controlled	Not controlled	None

Podisma pedestris	Arthropoda (Orthoptera)	Intra.	Hewitt et al., 1989	Both	Mating order	Not controlled	Not controlled	Analysis of deviance, logit link function
Chorthippus parallelus	Arthropoda (Orthoptera)	Intra.	Bella et al., 1992	One	Mating order	Not controlled	Not controlled	Chi-squared test, G test
Allonemobius fasciatus & *A. socius*	Arthropoda (Orthoptera)	Inter.	Howard & Gregory, 1993	Both	Mating order & number of spermatophores	Not controlled	Not controlled	None
			Gregory & Howard, 1994	Both	Mating order & number of spermatophores	Not controlled	Not controlled	G test
Tribolium castaneum & *T. freemani*	Arthropoda (Coleoptera)	Inter.	Wade et al., 1994	Both	Mating order & copulation rate	Accounted	Accounted	G test
			Robinson et al., 1994	NA	Mating order	Not controlled	Not controlled	None
			Fricke & Arnqvist, 2004	NA	Mating order & copulation time	Accounted	Accounted	Generalised linear model
Tribolium confusum	Arthropoda (Coleoptera)	Intra.	Wade et al., 1995	Both	Proportion of conspecific males	Not controlled	Not controlled	None
Drosophila simulans & *D. mauritiana*	Arthropoda (Diptera)	Inter.	Price, 1997	NA	Mating order & copulation time	Accounted	Accounted	None
			Turissini et al., 2018	Both	Mating order	Accounted	Not controlled	None
Drosophila simulans & *D. sechellia*	Arthropoda (Diptera)	Inter.	Price, 1997	NA	Mating order & copulation time	Accounted	Accounted	None
			Turissini et al., 2018	Both	Mating order	Accounted	Not controlled	None

Callosobruchus maculatus	Arthropoda (Coleoptera)	Intra.	Brown & Eady, 2001	Both	Mating order	Not controlled	Not controlled	Generalised linear model
Drosophila pseudoobscura	Arthropoda (Diptera)	Intra.	Dixon et al., 2003	Both	Mating order & copulation time	Not controlled	Accounted	Mann-Whitney U-test, squared-root transformation
Tribolium castaneum & *T. madens*	Arthropoda (Coleoptera)	Inter.	Fricke & Arnqvist, 2004	NA	Mating order & copulation time	Accounted	Accounted	Generalised linear model
Drosophila yakuba & *D. santomea*	Arthropoda (Diptera)	Inter.	Chang, 2004	One	Mating order & copulation time	Accounted	Accounted	Mann-Whitney U-test
			Turissini et al., 2018	One	Mating order	Accounted	Not controlled	None
Callosobruchus subinnotatus & *C. maculatus*	Arthropoda (Coleoptera)	Inter.	Rugman-Jones & Eady, 2007	NA	Mating order	Accounted	Accounted	Logistic regression
Chrysochus auratus & *C. cobaltinus*	Arthropoda (Coleoptera)	Inter.	Peterson et al., 2011	NA	Mating order	Not controlled	Not controlled	Mann-Whitney U-test
Gryllus bimaculatus & *G. campestris*	Arthropoda (Orthoptera)	Inter.	Tyler et al., 2013	NA	Mating order & spermatophore attachment time	Accounted	Accounted	Generalised linear mixed model
Teleopsis dalmanni	Arthropoda (Diptera)	Intra.	Rose et al., 2014	Both	Mating order	Accounted	Not controlled	Three-way nested ANOVA, Tukey's HSD test
Drosophila melanogaster & *D. simulans*	Arthropoda (Diptera)	Inter.	Castillo & Moyle, 2014	NA	Not controlled	Accounted	Not controlled	Logit model
			Turissini et al., 2018	Both	Mating order	Accounted	Not controlled	None

Drosophila erecta & *D. mauritiana*	Arthropoda (Diptera)	Inter.	Turissini et al., 2018	NA	Mating order	Accounted	Not controlled	None
Drosophila mauritiana & *D. melanogaster*	Arthropoda (Diptera)	Inter.	Turissini et al., 2018	Both	Mating order	Accounted	Not controlled	None
Drosophila mauritiana & *D. santomea*	Arthropoda (Diptera)	Inter.	Turissini et al., 2018	Both	Mating order	Accounted	Not controlled	None
Drosophila mauritiana & *D. sechellia*	Arthropoda (Diptera)	Inter.	Turissini et al., 2018	Both	Mating order	Accounted	Not controlled	None
Drosophila mauritiana & D. teissieri	Arthropoda (Diptera)	Inter.	Turissini et al., 2018	Both	Mating order	Accounted	Not controlled	None
Drosophila yakuba & *D. mauritiana*	Arthropoda (Diptera)	Inter.	Turissini et al., 2018	NA	Mating order	Accounted	Not controlled	None
Drosophila melanogaster & *D. santomea*	Arthropoda (Diptera)	Inter.	Turissini et al., 2018	NA	Mating order	Accounted	Not controlled	None
Drosophila melanogaster & *D. sechellia*	Arthropoda (Diptera)	Inter.	Turissini et al., 2018	Both	Mating order	Accounted	Not controlled	None
Drosophila melanogaster & *D. teissieri*	Arthropoda (Diptera)	Inter.	Turissini et al., 2018	NA	Mating order	Accounted	Not controlled	None
Drosophila santomea & *D. sechellia*	Arthropoda (Diptera)	Inter.	Turissini et al., 2018	Both	Mating order	Accounted	Not controlled	None
Drosophila simulans & *D. santomea*	Arthropoda (Diptera)	Inter.	Turissini et al., 2018	NA	Mating order	Accounted	Not controlled	None

Drosophila santomea & *D. teissieri*	Arthropoda (Diptera)	Inter.	Turissini et al., 2018	Both	Mating order	Accounted	Not controlled	None
Drosophila sechellia & *D. teissieri*	Arthropoda (Diptera)	Inter.	Turissini et al., 2018	NA	Mating order	Accounted	Not controlled	None
Drosophila simulans & *D. teissieri*	Arthropoda (Diptera)	Inter.	Turissini et al., 2018	NA	Mating order	Accounted	Not controlled	None
Drosophila yakuba & *D. teissieri*	Arthropoda (Diptera)	Inter.	Turissini et al., 2018	Both	Mating order	Accounted	Not controlled	None
Drosophila pseudoobscura & *D. persimilis*	Arthropoda (Diptera)	Inter.	Castillo & Moyle, 2019	NA	Not controlled	Accounted	Accounted	None
Drosophila montana	Arthropoda (Diptera)	Intra.	Garlovsky et al., 2020	One	Mating order	Accounted	Not controlled	Generalised linear model
Poecilia reticulata	Chordata (Cyprinodontiformes)	Intra.	Ludlow & Magurran, 2006	Both	Not controlled	Not controlled	Not controlled	Repeated measures ANOVA, arcsine transformation
Etheostoma hopkinsi & *E. luteovinctum*	Chordata (Perciformes)	Inter.	Mendelson et al., 2007	One	Sperm concentration	Accounted	Accounted	Binomial test on sperm-use probability
Etheostoma barrenense & *E. zonale*	Chordata (Perciformes)	Inter.	Williams & Mendelson, 2014	Both	Sperm concentration	Not controlled	Not controlled	None
Lepomis macrochirus & *L. gibbosus*	Chordata (Centrarchiformes)	Inter.	Immler et al., 2011	One	Sperm concentration	Accounted	Accounted	Binomial test on sperm-use probability
Salmo salar & *S. trutta*	Chordata (Salmoniformes)	Inter.	Yeates et al., 2013	Both	Not controlled	Accounted	Accounted	Paired t-test, square root

								arcsine transformation
Hyla versicolor & *H. chrysoscelis*	Chordata (Anura)	Inter.	Bogart, 1980	One	Sperm viability	Not controlled	Not controlled	NA (lack of variation)
Rana lessonae & *R. ridibunda*	Chordata (Anura)	Inter.	Berger & Rybacki, 1992	One	Not controlled	Not controlled	Not controlled	None
			Berger & Rybacki, 1994	One	Sperm concentration	Not controlled	Not controlled	None
Rana lessonae & *R. esculenta*	Chordata (Anura)	Inter.	Berger & Rybacki, 1992	NA	Not controlled	Not controlled	Not controlled	None
Mus musculus, *M. spretus* & *M. spicilegus*	Chordata (Rodentia)	Inter.	Martín-Coello et al., 2009	Both	Sperm concentration	Not controlled	NA (direct observation of fertilisation success)	None
Mus musculus & *M. domesticus*	Chordata (Rodentia)	Inter.	Dean & Nachman, 2009	One	Sperm concentration & viability	Not controlled	NA (direct observation of fertilisation success)	Fisher's exact test

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